

EFFECTS OF ADDING SUBSTRATES AND INHIBITORS ON THE HOMOGENATE RESPIRATION OF THE JAPANESE BEETLE, *POPILLIA JAPONICA* NEWMAN, DURING EMBRYONIC DEVELOPMENT*

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Ludwig and Barsa (1956) compared the respiration of 10 per cent homogenates of eggs of the Japanese beetle, *Popillia japonica*, and of the mealworm, *Tenebrio molitor*, with that of the whole eggs. They found that homogenization of newly laid Japanese beetle eggs resulted in a reduction of 50 per cent in O₂ consumption. From the third to the sixth days of embryonic development at 30°C., there was no reduction, but towards the end of the embryonic period, a reduction of more than 50 per cent was again obtained. They correlated the varying effects of homogenization on O₂ consumption with water content and the degree of organization of the egg. During the first 4 days of development at 30°C., the egg increases in weight from 0.83 to 2.3 mg., associated with the imbibition of water. During this time the constituents of the egg are greatly diluted and homogenization has little effect on respiratory rate. However, homogenization of the mealworm egg resulted in a reduction of approximately 48 per cent in O₂ consumption which was constant throughout the embryonic period at 30°C. Mealworm eggs do not imbibe water and the weight remains constant.

Ludwig and Barsa (1957) studied the effects of adding various substrates and inhibitors of the succinoxidase system to 5 per cent homogenates of the mealworm egg on its O₂ consumption for each day of embryonic development at 30°C. The addition of either cytochrome *c* or sodium succinate alone failed to increase respiratory rate. However, when both were added it was increased to that of the intact egg. The addition of the inhibitors, KCN, sodium malonate and DDT, when used singly, elimi-

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nated all of the O₂ uptake produced by the addition of substrates and a great deal of the endogenous respiration. Because of the physiological differences between the eggs of the mealworm and Japanese beetle noted above, it was decided to repeat this series of experiments using 5 per cent homogenates of Japanese beetle eggs.

MATERIAL AND METHODS

Eggs were collected daily so that when obtained they were always less than 24 hours old. They were kept in 1-ounce metal salve boxes containing moist soil at 30°C. At the desired stage, they were homogenized for one minute with a motor driven glass homogenizer in 0.03 molar phosphate buffer adjusted to a pH of 7.4. Readings on the O₂ consumption of 5 per cent homogenates were made over a 2-hour period in Warburg constant volume manometers at 30°C., according to the method outlined by Umbreit, Burris and Stauffer (1945). The substrates, sodium succinate and cytochrome *c* were added singly and together to the homogenate. The inhibitors, KCN, alcohol, or DDT in alcohol, were added singly to homogenates containing added sodium succinate and cytochrome *c*. All solutions were made in 0.03 molar phosphate buffer at a pH of 7.4. In each experiment, the thermobarometer contained all of the test solutions but no tissue homogenate. The final concentrations in the manometer flasks were:

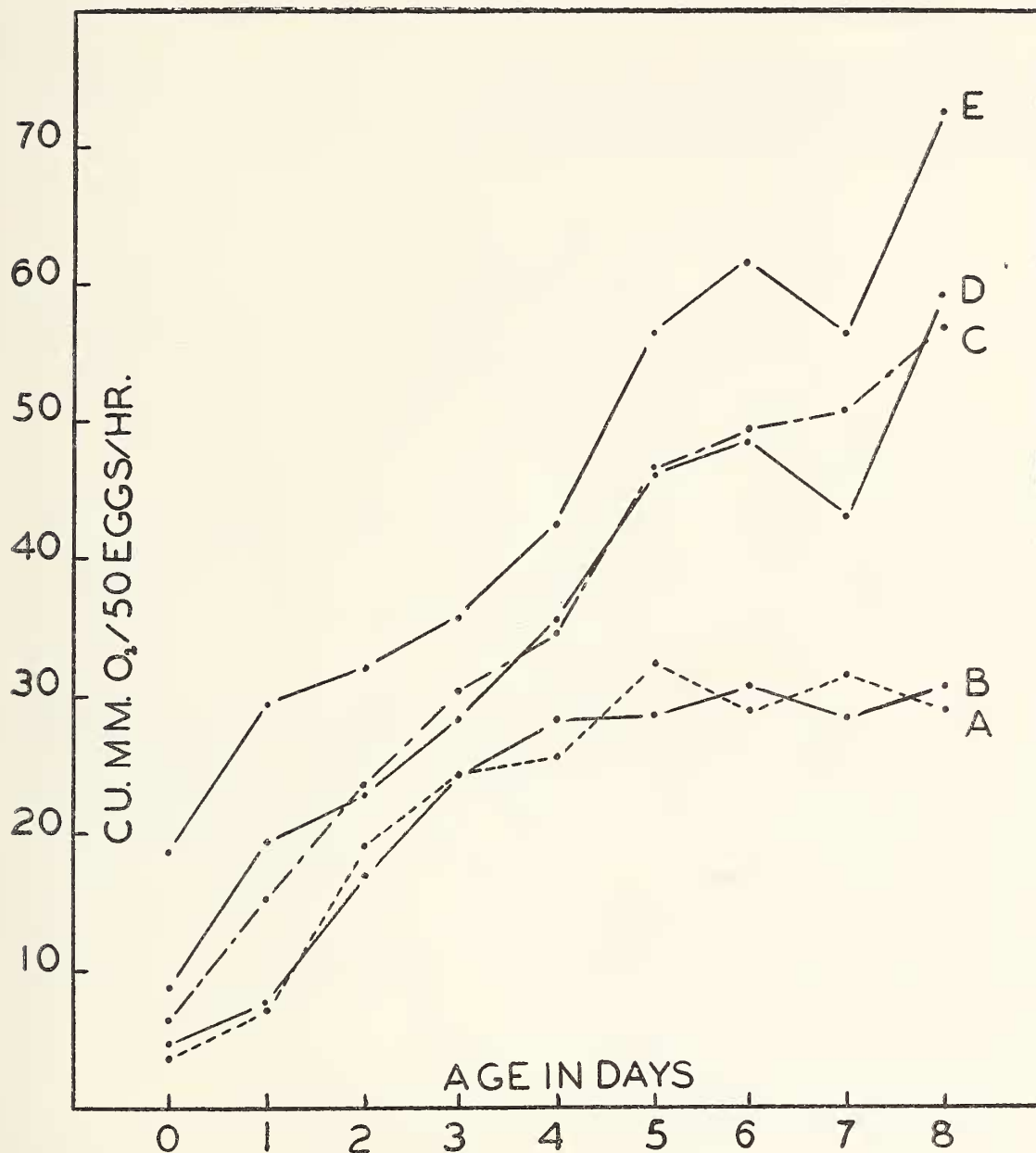
- (a) M/9 sodium succinate,
- (b) 0.6×10^{-4} M cytochrome *c*,
- (c) 10^{-4} M KCN,
- (d) 1.25 per cent ethyl alcohol,
- (e) 10^{-3} M DDT in 1.25 per cent ethyl alcohol.

The DDT was prepared by dissolving the crystals in 5 ml. of ethyl alcohol and adding this solution to 95 ml. of phosphate buffer. Cyanide inhibition was measured by the method of Robbie (1946). Throughout each experiment, the manometers were rocked horizontally 100 times a minute. Homogenates from the same individuals were used throughout one series of experiments.

OBSERVATIONS

The results are given in Figure 1. Each point is an average of at least 10 determinations. The O₂ consumption of unfortified homogenate is shown in Graph A. Endogenous metabolism

showed a progressive increase during the first 5 days of the embryonic period and then remained constant until the time of hatching. Sodium succinate (Graph C) and cytochrome *c*



EXPLANATION OF THE FIGURE

Figure 1. O₂ consumption of 5 per cent homogenates during embryonic development. Graph A, unfortified homogenate; Graph B, homogenates with cytochrome *c* and sodium succinate and with either KCN, 1.25 per cent alcohol, or DDT in 1.25 per cent alcohol; Graph C, homogenate with sodium succinate; Graph D, homogenate with cytochrome *c*; Graph E, homogenate with sodium succinate and cytochrome *c*.

(Graph D), when added singly, increased the rate of O₂ consumption to the same extent throughout the embryonic period. However, their stimulating effect was greatest during the latter part of this period when the rate of endogenous metabolism re-

mained constant. When both substrates were added simultaneously to the homogenate (Graph E), the increase in respiration was greater than with either substrate alone. The addition of KCN, alcohol, or DDT in alcohol to homogenates containing both substrates (Graph B) reduced the O₂ consumption to the level of the unfortified homogenate (Graph A). Each of the inhibitors decreased the respiratory rate to the same extent.

DISCUSSION

Since the addition of sodium succinate or of cytochrome *c* increased the respiration of homogenates of Japanese beetle eggs for each day of embryonic development, the enzymes of the succinoxidase system appear to be in excess throughout the embryonic period. The observation that respiration is enhanced during the second half of the egg stage to a greater extent than it is earlier, agrees with the results of Ludwig and Wugmeister (1955) that beginning with the 5-day egg at 30°C., the activities of cytochrome oxidase and succinic dehydrogenase increase rapidly until the end of the embryonic period.

Ludwig and Barsa (1957) found that the inhibitors, KCN or DDT when added to homogenates of mealworm eggs containing cytochrome *c* and sodium succinate, eliminated a great deal of the endogenous respiration. However, with Japanese beetle eggs, none of the endogenous metabolism was eliminated by these inhibitors. This observation indicates that a part of the endogenous metabolism of mealworm egg homogenates is mediated through the succinoxidase system; while in Japanese beetle eggs, the endogenous respiration is mediated entirely through a cyanide insensitive system such as a flavoprotein.

Ethyl alcohol (1.25 per cent) reduced the respiratory rate of homogenates of mealworm eggs, but its narcotic effect diminished towards the end of the embryonic period (Ludwig and Barsa, in press). The present experiments show that with homogenates of Japanese beetle eggs, the inhibitory effect of alcohol remained constant throughout embryonic development.

SUMMARY

The effects of adding substrates and inhibitors of the succinoxidase system on the O₂ consumption of 5 per cent homogenates were determined throughout the egg stage of the Japanese beetle at 30°C.

The addition of sodium succinate or cytochrome *c* stimulated respiration, the effect being greater during the second half of the egg stage. When both substrates were added simultaneously, the increase in respiration was greater than with either substrate alone.

KCN, alcohol, or DDT in alcohol, decreased the respiration of homogenates to which sodium succinate and cytochrome *c* had been added to the level of the unfortified homogenate. These inhibitors did not eliminate any of the endogenous respiration.

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